

## SPERMATOPHORE IN APHODIINAE (COLEOPTERA: SCARABAEIDAE)

IMELDA MARTÍNEZ M.

Departamento de Ecología y Comportamiento Animal, Instituto de Ecología A.C., Km. 2.5 Carretera Antigua a Coatepec 351, Congregación El Haya, Apartado Postal 63. 91070 Xalapa, Veracruz, México (e-mail: imelda@ecologia.edu.mx)

---

*Abstract.*—To study the spermatophore, females of three species, each of a different Aphodiini genus, and three species of one Eupariini genus we examined. All beetles were collected in the field throughout one reproductive cycle. In *Gonaphodiellus opisthius* (Bates) and *Planolinus vittatus* (Say) (Aphodiini), no spermatophore were found, probably because spermatozoa were freely transferred to the spermatheca in seminal fluid. In contrast, *Cephalocycclus hogei* (Bates) (Aphodiini), *Ataenius apicalis* Hinton, *Ataenius sculptor* Harold, and *Ataenius cribritorax* Bates (Eupariini) were found to form a spermatophore in transferring the spermatozoa. The spermatophore is formed by males during copulation. Its form and size are determined by the anatomy of the female genital chamber of each species. The spermatophore is a voluminous, vesicular structure, formed principally by a large quantity of secretions from the male accessory glands. Another small vesicle is found in the spermatophore's interior, containing secretions and the spermatozoa originating from the testes. The roles male secretions may play in female reproductive activity is discussed.

*Key Words:* spermatophore, Aphodiini, Eupariini

---

Limited studies have been done of reproductive anatomy, oviposition behavior, and reproductive cycles of Aphodiinae. These studies cover no more than 67 Aphodiini species and a few Eupariini and Psammodiini species (Martínez 2001, Martínez and Alvarado 2001, Martínez et al. 2001, Martínez and Cruz 2002, Cruz et al. 2002). Copulation has been observed superficially in four *Aphodius* species (Schmidt 1935, Landin 1961); in only one *Aphodius* species, *Aphodius distinctus*, has copulation been described in detail (Vitner 1995).

Neither the spermatophore nor functional aspects of the reproductive apparatus of either Aphodiinae sex is known. This paper provides the results of spermatophore studies in several species that belong to two tribes of this group, namely, Aphodiini y

Eupariini. This classification is to be used in this paper, even there are differences of opinion and some researchers regard the Aphodines, Euparines and Psammodynae as subfamilies of Aphodiidae (*sensu* Balthasar 1963).

### MATERIALS AND METHODS

Females of three species of three different genera of Aphodiinae (*sensu* G. Dellacasa et al. 2001, 2002) were examined during the reproductive cycle: 98 females of *Gonaphodiellus opisthius* (Bates) and 126 females of *Planolinus vittatus* (Say) (collected monthly from October 2000 to December 2001 in Las Vigas, Veracruz); and 102 females of *Cephalocycclus hogei* (Bates) (50 collected in June and July 2000 in Cuiyachapa, Veracruz, and 52 collected in June

Table 1. State of ovarian maturity and presence of spermatophore in the genital chamber of females of some Aphodiini and Eupariini species (n, number of females with spermatophore).

Species	Total Number of Females Examined	State of Sexual Maturity, and Presence of Spermatophore (n)
<b>Aphodiinae</b>		
<i>Cephalocyclus hogei</i>	102	Maturing <sup>(2)</sup> Mature <sup>(13)</sup>
<b>Eupariinae</b>		
<i>Ataenius apicalis</i>	130	Immature <sup>(4)</sup> Beginning maturation <sup>(9)</sup>
<i>Ataenius sculptor</i>	57	Immature <sup>(5)</sup> Beginning maturation <sup>(1)</sup>
<i>Ataenius cribrithorax</i>	30	Immature <sup>(2)</sup> Beginning maturation <sup>(2)</sup>

and July of 2001 in San José Aguazuelas, Veracruz).

In the Eupariini studies, we examined females of three species from the same genus: 130 *Ataenius apicalis* Hinton females (collected monthly from June 1998 to June 1999 in Los Lirios, Actopan, Veracruz); 57 *Ataenius sculptor* Harold females (collected in July 2000 in La Estancia, Palma Sola, Veracruz); and 30 *Ataenius cribrithorax* Bates females (collected in February 1999 in Los Tuxtlas, Veracruz).

All insects were collected directly from dung pats in grasslands in the aforementioned localities.

Reproductive systems of all females were placed in Ringer solution and were extended, fixed in AFATD (96% ethanol-formaldehyde-trichloroacetic acid-dimethylsulfide), and stored in 96% ethanol. Spermatocae were spread over slides by crumbling, fixed in AFATD, and stained with the Feulgen green light technique to determine whether spermatozoa were present. After fixation, the genital chambers with spermatophore were imbedded in Histosec<sup>®</sup> and the histological sections of 7  $\mu$ m were stained with Feulgen green light or hematoxylin-eosin. After fixation, reproductive systems were drawn to scale using camera lucida to determine the state of sexual maturity of each female (Martínez 2002). Testicular follicle smears were made, fixed

with methanol, and stained with Giemsa dye to observe the spermatozoa of each species.

## RESULTS

Spermatophores were not often found in the species studied, even though we examined a large number of females of each species over their entire reproductive periods. Spermatophores were found in only about 15% of one of the dissected Aphodiini species, *Cephalocyclus hogei*, and at comparable levels (10%–13%) in the three Eupariini species, *Ataenius apicalis*, *A. sculptor*, and *A. cribrithorax* (Table 1).

No spermatophore was found in any of the 98 *Gonaphodiellus opisthius* females or the 126 *Planolinus vittatus* females, regardless of maturational state. Apparently, these species do not form a spermatophore as a differentiated structure.

In each of these species, when the spermatophore was present, it completely filled the genital chamber. When the spermatophore was examined during dissection before fixation, it appeared as a voluminous, dense, whitish, refracting structure that distended the genital chamber wall, taking the form of this anatomical region according to the species (Fig. 1).

In all species examined, the spermatophore is seen microscopically as a large vesicle, delimited by an amorphous wall, at

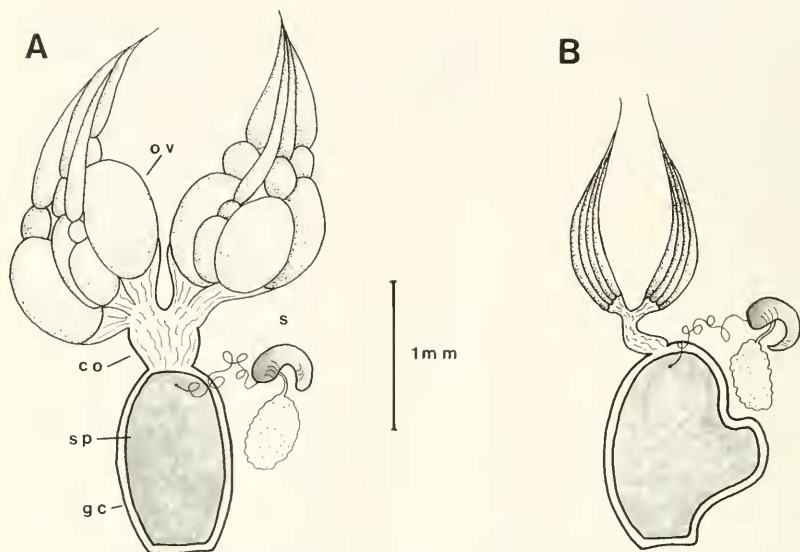


Fig. 1. Scheme of the spermatophore in the genital chambers. A. *Cephalocycclus hogei*. B. *Ataenius apicalis*. Abbreviations: co, common oviduct; gc, genital chamber; ov, ovary; s, spermatheca with its gland; sp, spermatophore.

times a very thick wall, depending on the species, which is contiguous with the epithelium of the genital chamber (Figs. 2A, B, C, D).

This vesicular structure holds a high concentration of secretions. Depending on the species, the secretions show greater affinities to different dyes, some to PAS +, others were acidophilic or basophilic. The secretions also show different forms: as larger or smaller platelets, or granular or amorphous structures, sometimes with vacuoles between them (Figs. 2A, B, C, D). Another much smaller vesicle is seen among the mass of secretions toward the anterior part of the genital chamber and close to the exit of the spermathecal duct. Within this smaller vesicle are found a smaller quantity of secretions and the spermatozoa. This smaller vesicle is more apparent in *Ataenius* species (Figs. 2B, C, D).

The spermatophore is probably formed

by the male during copulation, which in all the species studied takes place within a dung pat. Analysis of the glandular reservoirs of the males of the different species clearly shows morpho-functional modifications depending on state of sexual maturity, particularly relative to copulation. In immature males, the reservoirs are small and without secretions. In mature males before copulation, the reservoirs show their largest size and volume; and in males following copulation, the reservoirs decrease to roughly one-half (39%–67%) maximum volume, depending on the species (Fig. 3, Table 2).

During copulation, males liberate a large quantity of secretions from the accessory glands through the glandular ducts, secretions that have been stored in the glandular reservoirs. At the same time the males must liberate, through the vasa deferentia, the spermatozoa that come from the testicles.

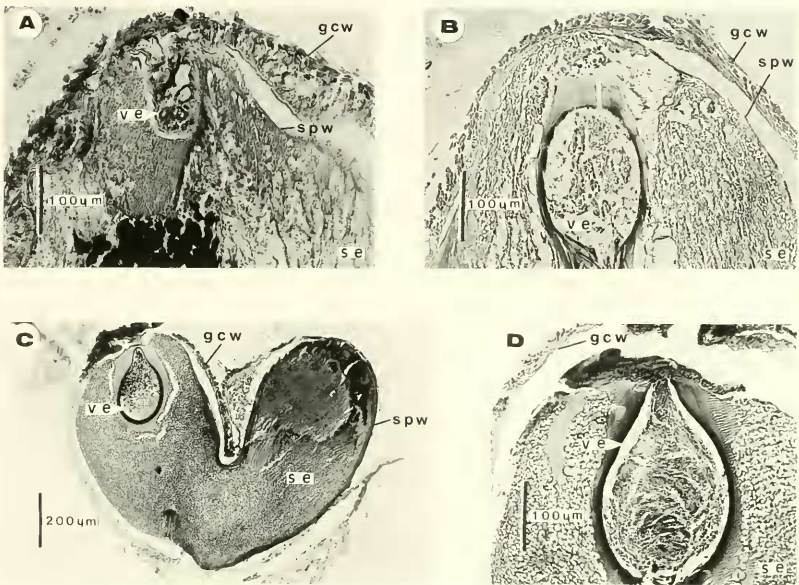


Fig. 2. Microphotographs of the spermatophore. A. *Cephalocyclus hogei*, partial view. B. *Ataenius apicalis*, partial view. C. *Ataenius cribritorax*, complete view. D. *Ataenius cribritorax*, partial view. Abbreviations: gcw, genital chamber wall; se, secretions; spw, spermatophore wall; ve, vesicle with secretions and spermatozoa.

Both the secretions and spermatozoa pass through the ejaculatory duct, found in the ejaculatory bulb, and from there through the internal sac of the aedeagus to be deposited in the female genital chamber as an elaborated spermatophore. In the six species examined, because the quantity of sper-

matozoa was low, morpho-functional changes in the testicles were not dramatic.

Spermatozoa size varied depending on the species. In Aphodiini, spermatozoa are long, though the length varies according to the species' follicle size. In *Gonaphodiellus opisthius*, males have seven follicles of the

Table 2. Variation in male glandular reservoir volume of some Aphodiini and Eupariini species in different states of sexual maturity (n, number of males examined).

State of Maturity	Glandular Reservoir Volume ( $10^{-3} \text{ mm}^3$ ), $\bar{x} \pm \text{SD}$ , (n)		
	<i>Cephalocyclus hogei</i>	<i>Ataenius apicalis</i> *	<i>Ataenius vulpor</i> *
Immature	$23.83 \pm 14.11^{(9)}$	$1.03 \pm 0.32^{(5)}$	$1.46 \pm 1.08^{(18)}$
Mature			
Before copulation	$482.66 \pm 71.04^{(6)}$	$46.50 \pm 0.70^{(6)}$	$123.16 \pm 5.0^{(3)}$
After copulation	$188.83 \pm 49.38^{(11)}$	$22.64 \pm 3.30^{(15)}$	$81.80 \pm 6.0^{(3)}$

\* Because of the anatomy of *Ataenius* species, the volume calculated for these species was that of the sacs of the glandular reservoirs.

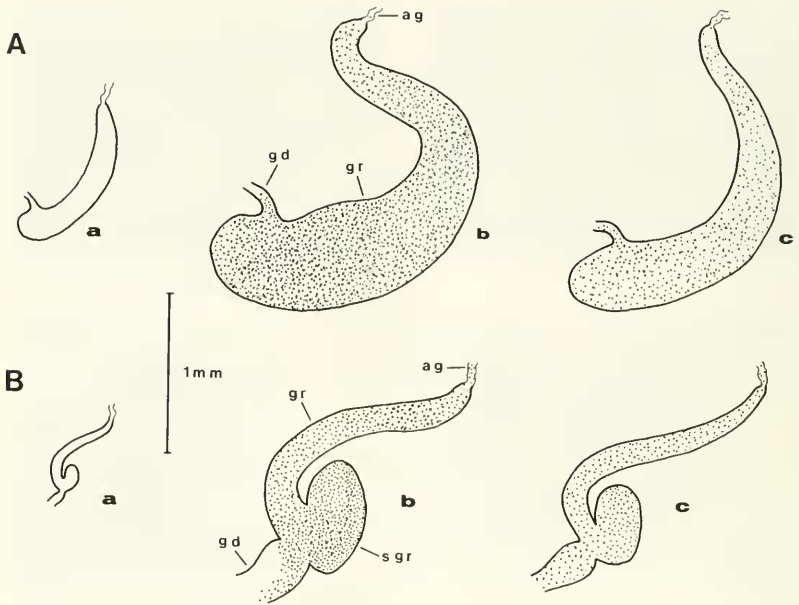


Fig. 3. Scheme of the male glandular reservoirs. A, *Cephalocyclus hoguei*. B, *Ataenius apicalis*. a, immature; b, mature before copulation; c, mature after copulation. Abbreviations: ag, accessory gland termination; gd, beginning of the glandular duct; gr, glandular reservoir; se, secretions; sgr, sac of the glandular reservoir.

same size and the spermatozoa measure up to 2,000  $\mu\text{m}$ . In contrast, *Planolinus vittatus* has six large follicles and two small ones, and two sizes of spermatozoa, 600 and 1,100  $\mu\text{m}$ . *Cephalocyclus hoguei* has three large follicles and two small ones, and two spermatozoa sizes, 800 and 1,500  $\mu\text{m}$ . The three Eupariini species, *Ataenius apicalis*, *A. sculptor*, and *A. cribritorax*, each have just two testicular follicles of the same size per testis, and the size of their spermatozoa does not vary so much, from 100 to 160  $\mu\text{m}$ , depending on the species.

The presence of a spermatophore and state of ovarian maturity differed among the species studied (Table 1). In *C. hoguei*, the spermatophore was not present in any immature female, but it was found in maturing females with several oocytes per ovariole as

well as in mature females before and after oviposition. In *A. apicalis*, *A. sculptor*, and *A. cribritorax*, the spermatophore was found in the genital chamber in immature females without oocytes and in females that had begun maturation (i.e., that presented a very small basal oocyte in each ovariole).

In all species studied, including *G. opisthius* and *P. vittatus*, the females toward the end of maturation or at maturity had spermatozoa in the spermatheca. This finding suggests that once immature or mature females have copulated, the spermatozoa pass to the spermatheca, while the secretions that form the spermatophore may be reabsorbed in the genital chamber. In *G. opisthius* and *P. vittatus*, in which spermatophores were never found, the spermatozoa must be freely transferred in the seminal

fluid to the genital chambers of maturing females and from there to the spermatheca. In these species, it is very likely that the male secretions forming the seminal fluid are also reabsorbed in the genital chamber.

#### DISCUSSION

The form and size of spermatophore in *Cephalocycclus hoguei*, *Ataenius apicalis*, *A. sculptor*, and *A. cribritorax* conforms to the genital chamber of each species, as in other Coleoptera species (Landa 1960, Cruz and Martínez 1992).

The spermatophore of these species is vesicular, as is also true for Coleoptera species belonging to other families (Fisher 1959, Gundevia and Ramamurty 1977, Obata 1987). In contrast, in the seven Scarabaeidae species that have been studied, this structure is more elaborate—a vesicular-filamentous structure with a very long filamentous structure (Heymons 1930, Halfpiter and López 1977, Halfpiter et al. 1980, Cruz and Martínez 1992).

In insects, particularly Coleoptera, the transfer of spermatozoa to the spermatheca is effected in two principal ways: through the initial formation of a spermatophore, which is transferred to the genital chamber, where it is reabsorbed, toward liberating the spermatozoa; or through the free transfer of spermatozoa in seminal fluid (Davey 1960, Mann 1984). *Cephalocycclus hoguei*, *Ataenius apicalis*, *A. sculptor*, and *A. cribritorax* belong to the former group, and *Gonaphodiellus opisthius* and *Planolinus vittatus* to the latter.

The secretions that form the spermatophore in *C. hoguei*, *A. apicalis*, *A. sculptor*, and *A. cribritorax* come from the accessory glands of the male, and the chemical nature of these secretions is unknown. The accessory glands of these species are very developed mesadenes, there are not ectadenes (Martínez et al. 2001). It has been widely observed that mesadenes in insects are the principal glands involved in spermatophore formation (Leopold 1976, Mann 1984).

The abundant secretions of the spermatophore of the species studied are no doubt

involved in other reproductive processes. In insects, these secretions are responsible for inducing complex functions, such as intervening in the formation of the spermatophore, providing energy to maintain and transport spermatozoa in the female genital tract, inducing ovarian maturation and vitellogenesis, and even forming part of the oocyte yolk (Mann 1984, Huignard 1984, Martínez and Cruz 1999).

It is very likely that, in the species examined here, the secretions play an important role in ovarian maturation given that copulation is carried out with females that are immature or just beginning maturation, and not with those that are mature, as is the case for other species of insects (Raabe 1986), particularly some Scarabaeidae species (Martínez and Cruz 1990, Martínez et al. 1996). On the other hand, in *Cephalocycclus hoguei*, copulation occurs when the females are mature. One possible explanation is that, because *C. hoguei* has a very short reproductive cycle following a very long diapause (Cruz et al. 2002), the spermatophore secretions may terminate vitellogenesis such that oviposition may occur as quickly as possible. *Ataenius apicalis* and *A. sculptor*, in contrast, present longer reproductive cycles, particularly the first, which shows no diapause (Martínez and Cruz 2002). The cycle of *A. cribrithorax* has not yet been documented.

The fact that relatively few spermatozoa were seen in the spermatophore of the six Aphodiinae species studied is consistent with the studies of Virkki (1951, 1957). Virkki mentions that the number of spermatogonia in the germarium is low, with the result that fewer cysts are present during spermatogenesis and in turn fewer spermatozoa are seen at the end of the process.

The unusual spermatozoa size found in Aphodiini species has been observed in other Coleoptera species, including *Alogosia bicolor* (L.) (Chrysomelidae) (Virkki and Bruck 1994), *Divalves bipustulatus* (F.) (Cleridae), and *Pinella aptera* (Guerin)

(Ptiliidae) (Mazzini 1976, Taylor et al. 1982), and in some Diptera species, including *Drosophila littoralis* (Drosophilidae) Meigen (Bressac et al. 1991). What would be the advantage to Aphodiini species to have such a small number of long spermatozoa? Several other questions also arise about spermatozoa size: How do these spermatozoa pass from the testes to the spermatheca? And afterward, how are the spermatozoa released from the spermatheca to fertilize the oocyte?

This spermatozoa behavior during and after copulation is not known, nor is much else known about the reproductive strategies of dung beetles, in particular Aphodiinae species, which have been the least studied.

#### ACKNOWLEDGMENTS

This work was carried out with the help of the Departamento de Ecología y Comportamiento Animal of the Instituto de Ecología A. C. (Clave 902-11). I am grateful for the valuable field and laboratory technical help provided by Julien Passerat, Teresa Suárez, Tania Pensado, Mayvi Alvarado and Magdalena Cruz R., to Ann Covault for the English translation and two anonymous reviewers for their comments on the manuscript.

#### LITERATURE CITED

- Balthasar, V. 1963. Monographie der Scarabaeidae und Aphodiidae der Palearktischen und orientalischen Region. Coleoptera: Lamellicornia. III Aphodiidae. Tschechoslowakischen Akademie der Wissenschaften, Prague, 652 pp.
- Bressac, C., D. Joly, J. Devaux, and D. Lachaise. 1991. Can we predict the mating pattern of *Drosophila* females from the sperm length distributions in males? *Experientia* 47:11–114.
- Cruz, R. M. and M. I. Martínez. 1992. Estructura y formación del espermatoforo en *Canthon Hoffmannsegg* (Coleoptera: Scarabaeidae). *Elytron* 6: 119–131.
- Cruz, R. M., M. I. Martínez, and O. M. Alvarado. 2002. Population and reproductive features of *Aphodius* (*Trichaphodius*) *opisthius* Bates and *Cephalocyclus hoguei* Bates (Coleoptera, Aphodiidae: Aphodiinae). *The Coleopterists Bulletin* 56(2): 221–235.
- Davey, K. G. 1960. The evolution of spermatophores in Insects. *Proceedings of the Royal Entomological Society of London* 35: 107–113.
- Dellacasa, G., P. Bordat, and M. Dellacasa. 2001. A revisional essay of world genus-group taxa of Aphodiinae (Coleoptera Aphodiidae). *Memorie della Società Entomologica Italiana* 79: 1–482.
- Dellacasa, M., R. D. Gordon, and G. Dellacasa. 2002. Aphodiinae described or recorded by Bates in Biología Centrali-Americana (Coleoptera Scarabaeoidea: Aphodiidae). *Acta Zoológica Mexicana* (n.s.) 86: 155–223.
- Fisher, T. W. 1959. Occurrence of spermatophores in certain species of *Chilocorus* (Coleoptera: Coccinellidae). *Pan-Pacific Entomologist* 35: 205–208.
- Gundevia, H. S. and P. S. Ramamurty. 1977. The male accessory reproductive glands and spermatophore in *Hydrophilus olivaceus* (Polyphaga-Coleoptera). *Zeitschrift für Mikroskopische-Anatomische Forschung, Leipzig* 91(3): 475–492.
- Halfpiter, G. and Y. López-G. 1977. Development of ovary and mating behavior in *Phanaeus*. *Annals of Entomological Society of America* 70(2): 203–213.
- Halfpiter, G., V. Halfpiter, and C. Huerta. 1980. Mating and nesting behavior of *Eurysternus* (Coleoptera: Scarabaeinae). *Quaestiones Entomologicae* 16: 599–620.
- Heymons, R. 1930. Über die Morphologie des Weiblichen Geschlechtsapparats der Gattung *Scarabaeus* L. *Zeitschrift für Morphologie und Ökologie der Tiere* 18: 536–574.
- Hugnard, J. 1984. Transfer, importance physiologique et spécificité des sécrétions mâles chez les femelles d'*Acanthoscelides obtectus* Say. *Bulletin de la Société entomologique de France* 89: 953–961.
- Landa, V. 1960. Origin, development and function of the spermatophore in cockchafer (*Melolontha melolontha* L.) *Acta Societatis Entomologicae Cechoslovenicae* 57(4): 297–315.
- Landin, B. 1961. Ecological studies on dung beetles. *Opuscula Entomologica*, Sup. 19: 1–172.
- Leopold, R. A. 1976. The role of male accessory glands in insect reproduction. *Annual Review of Entomology* 21: 199–221.
- Mann, T. 1984. Spermatophore development, structure, biochemical attributes and role in the transfer of spermatozoa. *Zoophysiology* 15: 1–217.
- Martínez, M. I. 2001. La biología reproductiva en Aphodiidae: Síntesis de datos conocidos (Coleoptera). *Bolletino della Società Entomologica Italiana* 133(2): 133–130.
- . 2002. Técnicas básicas de anatomía microscópica y de morfometría para estudiar los insectos. *Boletín de la Sociedad Entomológica Aragonesa* 30: 187–195.
- Martínez, M. I. and O. M. Alvarado. (2001) Comportamiento de oviposición en *Aphodius opisthius*

- Bates 1887 (Coleoptera: Scarabaeoidea: Aphodiidae). *Elytron* 15: 73–78.
- Martínez, M. I. and R. M. Cruz. 1990. Cópula, función ovárica y nidificación en dos especies del género *Canthon* Hoffmannsegg (Coleoptera: Scarabaeidae). *Elytron* 4: 161–169.
- . 1999. The effects of male glandular secretions on female endocrine centers in *Canthon cyanellus cyanellus* LeConte (Coleoptera: Scarabaeidae, Scarabaeinae). *The Coleopterists Bulletin* 53(3): 208–216.
- . 2002. Fenología y ciclos reproductivos en *Ataenius apicalis* Hinton y *Ataenius sculptor* Harold (Coleoptera Scarabaeoidea Aphodiidae: Eupariinae). *Bulletin de la Société entomologique de France* 107(2): 177–186.
- Martínez, M. I., L. C. Deloya, and M. Dellacasa. 2001. Anatomical and functional data on female and male reproductive systems of some dung beetle species of Aphodiinae and Eupariinae of Mexico (Coleoptera: Scarabaeoidea: Aphodiidae). *Proceedings of the Entomological Society of Washington* 103(1): 22–248.
- Martínez, M. I., C. Huerta, and R. M. Cruz. 1996. Comportamiento reproductor en hembras de *Coprins incertus* Say (Coleoptera, Scarabaeidae). *Bulletin de la Société entomologique de France* 101(2): 121–130.
- Mazzini, M. 1976. Giant spermatozoa in *Divalves bipustulatus* F. (Coleoptera: Cleridae). *International Journal of Insect Morphology and Embryology* 5: 107–115.
- Obata, S. 1987. Mating behavior and sperm transfer in the ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). *Applied Entomology and Zoology* 22(4): 434–442.
- Raabe, M. 1986. Insect reproduction: Regulation of successive steps. *Advances in Insect Physiology* 19: 29–154.
- Schmidt, G. 1935. Beiträge zur Biologie des Aphodiinae. *Stettiner Entomologische Zeitung* 96: 293–350.
- Taylor, V. A., B. M. Luke, and M. B. Lomas. 1982. The giant sperm of a minute beetle. *Tissue and Cell* 14: 113–123.
- Virkki, N. 1951. Zur Zytologie einiger Scarabaeiden (Coleoptera). Studien an der Spermatogenese. *Annales Zoologici Societatis Zoologicae Botanicae, Fennicae "Vanamo"* 14: 1–104.
- . 1957. Structure of the testis follicle in relation to evolution in the Scarabaeidae (Coleoptera). *Canadian Journal of Zoology* 35: 265–277.
- Virkki, N. and T. Bruck. 1994. Unusually large sperm cells in Alticidae: their formation and transportation in the male genital system, and their evolution, pp. 371–381. In Jolivet, P. H., M. L. Cox, and E. Petitpierre, eds. *Novel Aspects of the Biology of Chrysomelidae*. Kluger Academic Publishers, Dordrecht, Netherlands.
- Vitner, J. 1995. Mating behavior of *Aphodius (Chlothorax) distinctus* (Coleoptera: Scarabaeidae: Aphodiinae). *Acta Societatis Zoologicae Bohemicae* 59: 249–265.